Translation Inhibitors

Introduction

These antibiotics affect translation. Translation occurs in the cytoplasm. To get from the extracellular space into the cytoplasm, the drug has to get through a few defenses. For Gram-positive organisms, it has to get through a thick peptidoglycan cell wall and through one plasma membrane. For Gram-negative organisms, it has to get through two plasma membranes, and a small peptidoglycan cell wall in between. Because ribosomal antibiotics get through a plasma membrane in both cases, and don't do anything unless in the cytoplasm, both Gram-positive and Gram-negative organisms can develop mutations that prevent the entry of the drug (reduced penetration through porins) and mutations that increase its efflux through pumps. The resistance mechanisms we saw in β-lactams don't work against these drugs—they aren't bothering with the peptidoglycan cell wall construction; they're breaking ribosomal translation. And the DNA that codes for ribosomes is highly conserved. Mutating the ribosome to evade the drug isn't going to happen. Making other proteins that bind up or inactivate the drug, so the drug can't do anything to the ribosome, can happen.

There are many classes and drugs in this lesson. Your goal is to know whether they attach to the 30s or the 50s ribosome and which step of translation they mess up. This is an excellent opportunity to force you to know pharmacology—antibiotic mechanisms, indications, and side effects—then test your biochemistry knowledge—steps of translation. Yes, you will a have question in the challenge section specifically about biochemistry. Just one, though.

We start off with a brief review of translation, then progress through the drug classes and individual antibiotics in order of the events of translation. We organize the lesson according to Table 4.2. Inhibition of protein synthesis is universally **bacteriostatic** except for the aminoglycosides, which can be bactericidal by causing misreading of RNA.

Brief Review of Bacterial Translation

There is no nucleus in a bacterium. The DNA is transcribed into RNA at the same time that RNA is transcribed by ribosomes into amino acids. The 50s and 30s ribosomal subunits assemble on the RNA to form the 70s ribosomal unit. All tRNAs in this discussion have already been activated—the tRNA with the anticodon appropriately attached to the amino acid at the 3' terminus of the tRNA strand.

Initiation. Ribosomes read codons, three-nucleotide-long strings of RNA. Ribosomes assemble at the start codon, AUG, which codes for methionine. A tRNA carrying methionine binds its anticodon to the codon AUG. This stimulates the arrival of the ribosomal subunits. The ribosome assembles so that the tRNA-met starts at the P site. The only tRNA anticodon to bind to the P site is the tRNA that carries methionine. But it does not BIND TO the P site. The tRNA anticodon is already bound to its mRNA codon and defines where the ribosome forms. With assembly complete, translation only begins with when there is the signal to initiate—initiating factor. With assembly + initiating factor, the empty A site accepts the tRNA-amino acid with the anticodon that matches the codon of the mRNA at the A site. Peptidyl transferase moves the methionine from the P site to the amino acid at the A site. Methionine's tRNA does not move. Initiation has completed.

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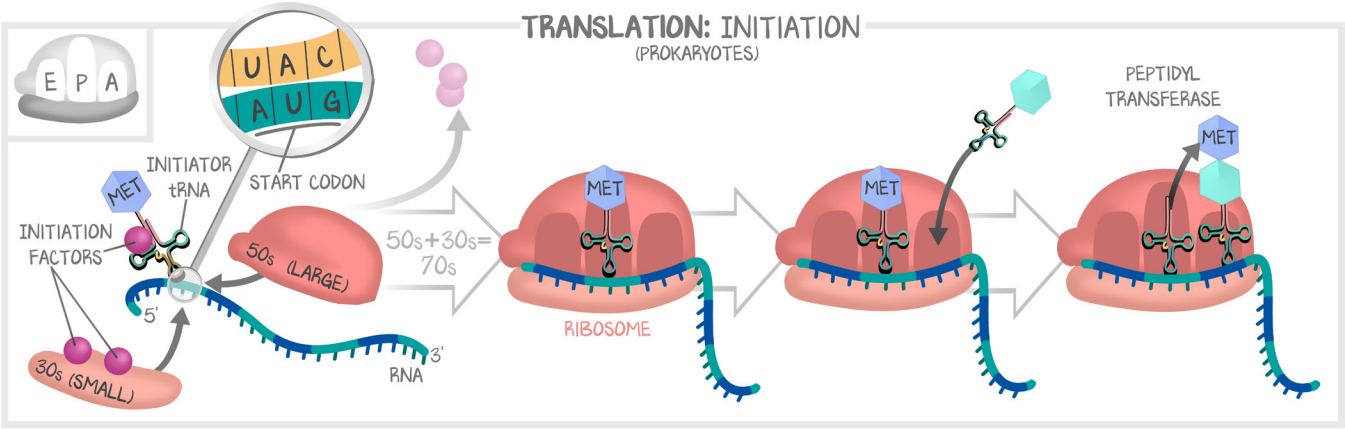

Figure 4.1: Review of Initiation
A tRNA carrying methionine (met) identifies the start codon AUG. This signals the formation of the ribosome, the 30s and 50s subunits combining onto the RNA such that the Met-tRNA is in the P site. Initiation factors are required for the A site to accept the first amino acid to be added and the first peptidyl transferase reaction ensues.

Elongation. In order to elongate, elongation factor is required. When elongation factor instructs the ribosome to move, the ribosome translocates one codon forward. The tRNA that held the methionine doesn't move, its tRNA's anticodon still bound to the RNA codon. But translocation has moved the ribosome so now that tRNA is in the exit site relative to the ribosome. The ribosome allows the anticodon and codon to separate, and the tRNA without an amino acid is ejected. The tRNA holding the amino acid–methionine chain doesn't move either, its tRNA's anticodon still bound to the RNA codon. But the ribosome translocated, so now that strand is in the P site relative to the ribosome. There was nothing bound to the RNA when the ribosome translocated to what is the new A site—it is empty. The third amino acid enters the A site, its tRNA's anticodon binding to the RNA codon at the A site. The amino acid chain is moved to the newest amino acid chain by peptidyl transferase. This process repeats until termination.

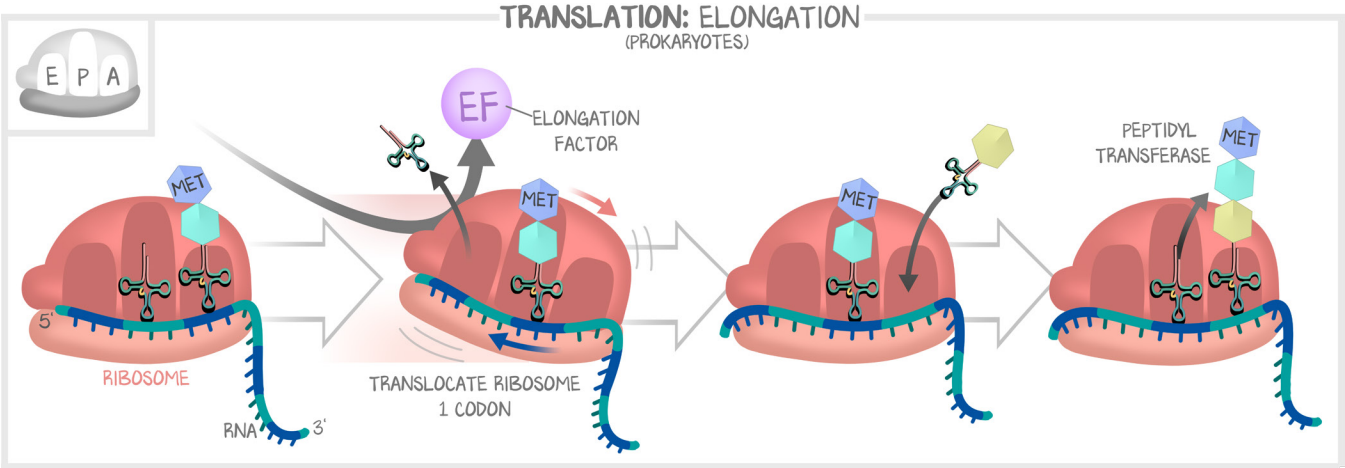

Figure 4.2: Review of Elongation
With the first peptide transfer to the A site, elongation factor ensures the translocation of the ribosome one codon forward, moving three nucleic acids, expelling the spent tRNA from the E site, opening the A site for the next tRNA..

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Termination. A stop codon is encountered. Peptidyl transferase, the enzyme that moves the amino acid chain from the P site tRNA to the A tRNA, now hydrolyzes the final amino acid sequence from the tRNA and releases the amino acid sequence. Ribosome subunits dissociate.

There are no drugs that affect initiation factor, elongation factor, or termination. At least that you have to know about.

Overview

Antibiotics that interfere with protein translation can bind to the 50s ribosome or the 30s ribosome. Antibiotics that interfere with protein translation can inhibit the formation of the initiation complex (inhibition of initiation), inhibit the A site codon's binding to its tRNA anticodon, inhibit peptidyl transferase, or prevent translocation.

LOCATION OF BINDING	CLASS	MECHANISM
50s	Macrolides, clindamycin	Inhibits translocation
	Streptogramins	Inhibits elongation
	Chloramphenicol	Inhibits peptidyl transferase
	Linezolid	Inhibits initiation complex
30s	Aminoglycosides	Prevents assembly of 70s (-static) Misreading of DNA (-cidal)
	Tetra-cyclines	A-site, prevents addition of charged tRNA
	Glycyl-cyclines	Same as tetracyclines

Table 4.1: Class and Mechanism According to Binding Location

EVENT	ANTIBIOTIC	MECHANISM
Formation of initiation complex	Aminoglycosides (30s)	Binds to the 30s ribosome, distorts the frame Warped P site before formation = no initiation Warped A site during translation = misreading = bactericidal
	Linezolid (50s)	Inhibits initiation complex Does not warp the A site, so does not cause misreading
Formation of peptide bond	Chloramphenicol (50s)	Inhibits peptidyl transferase
A-site addition of charged tRNA	Tetracyclines (30s)	Block the A site, new aminoacyl tRNA cannot be accepted, each class in a slightly different way
	Glycylcyclines (30s)	
	Streptogramins (50s)	
Translocation	Macrolides (50s)	Bind to ribosome and prevent the translocation step
	Clindamycin (50s)	
Elongation Factor	None	N/A
Initiation Factor	None	N/A
Termination	None	N/A

Table 4.2: Antibiotics and Their Corresponding Mechanisms by Event

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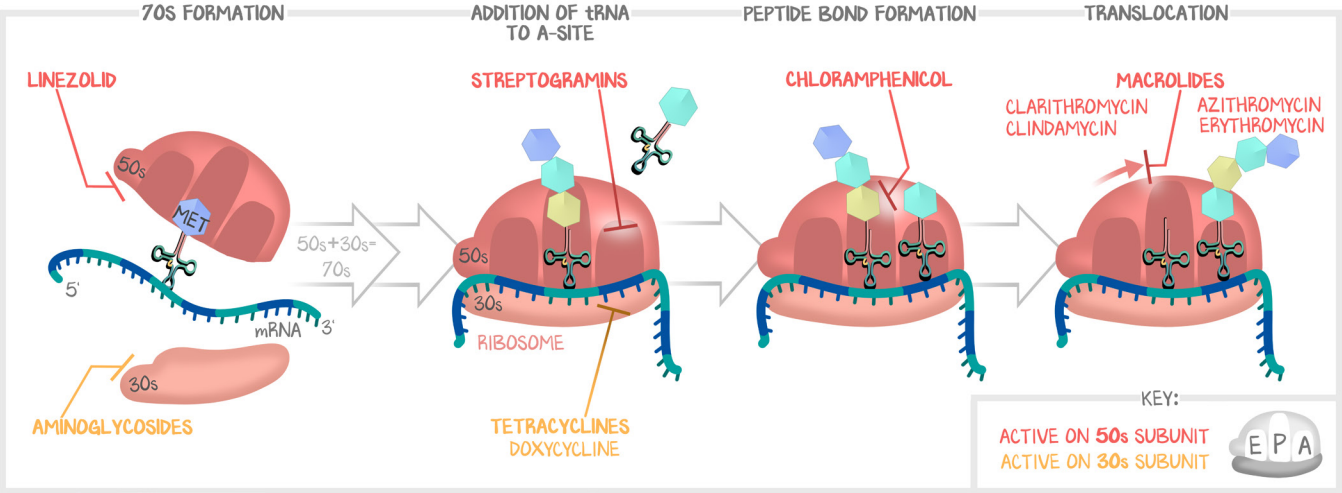

Figure 4.3: Mechanisms of Drugs That Work on Protein Synthesis
Numerous mechanisms inhibit prokaryotic translation. Linezolid and aminoglycosides inhibit the formation of the 70s. Streptogramins and tetracyclines inhibit the addition of an incoming tRNA to the open A site. Chloramphenicol inhibits the transfer of the amino acid chain to the incoming amino acid. Macrolides inhibit translocation. This image is organized similar to the whiteboard plan, showing the orientation of 30s mechanisms on the bottom, 50s mechanisms on the top.

Aminoglycosides

Aminoglycosides were once used as the empiric therapy for Gram-negative infections. The combination of ampicillin and gentamycin was a regular option for Gram negatives. The reason we chose that combination is because β-lactams and aminoglycosides are **synergistic**—the β-lactams clear the peptidoglycan layer, which allows the aminoglycoside entry to the cell. Aminoglycosides are so toxic that they rarely are used anymore, except inhaled tobramycin in cystic fibrosis. Since so many classes of drugs end with -mycin or -cin, the nomenclature is useless. The examples you should be able to recognize as aminoglycosides are *gentamicin, amikacin, tobramycin, streptomycin*. "Gent" (Gram-negative infections) and "tobra" (inhaled, cystic fibrosis) you will see more often than the others in life. **Only aminoglycosides are bactericidal** in this lesson.

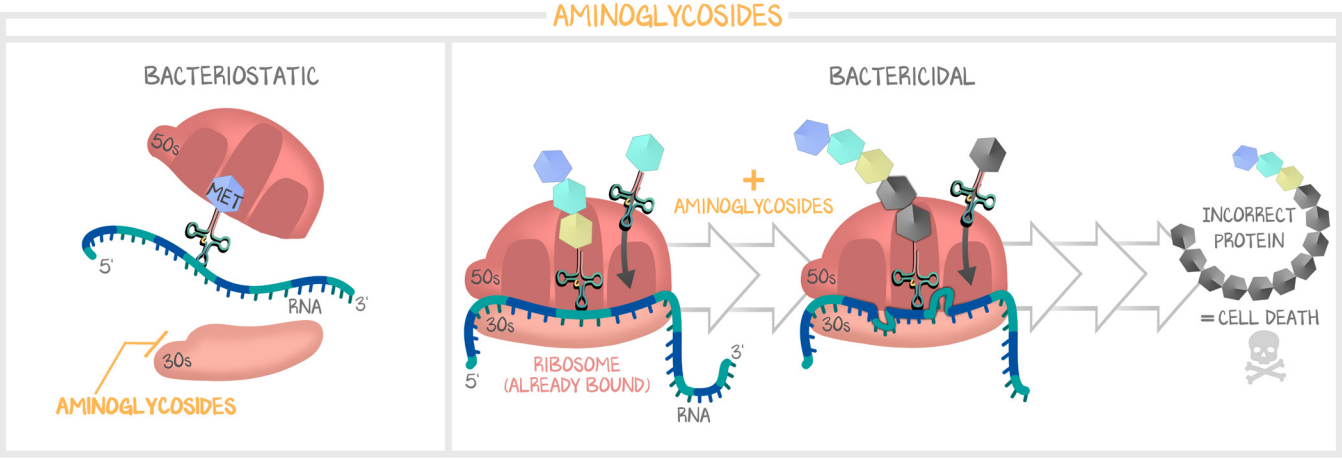

Figure 4.4: Aminoglycosides
Aminoglycosides are the only translation inhibitor that can be bactericidal. Inhibition of the 30s subunit of an unformed 70s ribosome is bacteriostatic because it prevents protein translation. Binding of the aminoglycoside to the 30s subunit of an already translating 70s ribosome causes misreading of the RNA, resulting in the synthesis of erroneous proteins. Without a mechanism to clear these proteins, they accumulate in the cytoplasm, leading to cell death.

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Mechanism. Aminoglycosides gain access to the cytoplasm by passing through porins in the outer membrane (Gram-negative requirement only) then through the inner membrane via **oxygen-dependent transport mechanisms**. Because their entry mechanism requires oxygen, by definition they cannot have an effect on anaerobes. Once in the cell, they bind to the **30s ribosomal subunit**. This does one of two things. For a 30s ribosome that has not yet formed an initiation complex, it **prevents assembly**, preventing the initiation of translation, which is bacteriostatic. Bind the 30s, no new assemblies can be made, and the cell runs out of protein. For a 30s subunit of an actively translating ribosome, it causes **misreading of the code**, resulting in an **aberrant protein**, which hastens cell death, which is bactericidal. Accumulation of bad proteins in the cytoplasm causes other processes to go awry. Bacteria have no good way to remove bad proteins, nor a vesicle to stash misfolded proteins in, so when protein misfolds occur in great number (as when the cytoplasm is saturated with aminoglycoside), the cell dies. This effect is **concentration dependent**, such that the goal is to achieve a maximum concentration that is many times the minimum inhibitory concentration—aminoglycosides are not time-above-the-line as penicillins were; they are more effective the higher the dose. And they exhibit a **prolonged postantibiotic effect** in that they continue to be effective even after their concentration falls below the mean inhibitory concentration.

Side effects. The main side effects of aminoglycosides are ototoxicity and nephrotoxicity. As well as aminoglycosides kill bacteria at high concentrations, so too do they kill ear and kidney cells. Accumulation of aminoglycoside in the endolymph and perilymph will destroy the hair cells of the organ of Corti (Neuroscience: Special Senses #4: *The Ear: Audition and Balance*). Reabsorption of aminoglycosides in the proximal tubule of the nephron disrupts intracellular calcium balance, which is toxic to the cells. **Ototoxicity** is related to the dose and duration of therapy (accumulation). **Nephrotoxicity** causes acute tubular necrosis because of toxic concentrations in the nephron. Giving fluids can mitigate nephrotoxicity—diluting the urine and increasing the flow decreases the time and concentration at which the toxic drug is exposed to any one cell. Because furosemide also causes ototoxicity and nephrotoxicity, it should never be used in conjunction with aminoglycosides. One could erroneously deduce that use of a loop diuretic such as furosemide would "flush the kidney," as fluids do. While more urine is produced in the bladder, loop diuretics serve only to concentrate the blood—just as much drug and fluid are filtered, but less water is reabsorbed, INCREASING toxic effects.

Elimination. They are renally eliminated, water soluble, and **do require** dose adjustment for renal failure.

Resistance. To get into the cytoplasm, aminoglycosides must use membrane channels, such as porins. Mutating the porin could deny entry of the drug to the cell. Effusion pumps at the inner membrane could knock it out into the periplasmic space. Since ribosomes cannot mutate (they are conserved) and the drug is in the cytoplasm, bacteria have learned to inactivate aminoglycosides. Bacterial transferase enzymes lead to **aminoglycoside inactivation** via acetylation, phosphorylation, or adenylation.

Linezolid

Linezolid **inhibits the formation of initiation complex** as well, but does it **from the 50s side**. It binds to the **23s** portion of the 50s only while unassociated from the 30s. This prevents the formation of the 70s and therefore prevents initiation. Unlike aminoglycosides, linezolid does NOT cause misreading of DNA, so is only bacteriostatic. Linezolid is used in to treat **vancomycin-resistant** organisms, particularly *Staph. aureus* (VRSA) or **vancomycin-resistant enterococcus** (VRE). In comparison to daptomycin (see Antibiotics #2: *β-Lactam Cell Wall Inhibitors*), it **can treat staph pneumonia** but should **NOT be used to treat staph bloodstream infections**. Linezolid comes in both oral and intravenous preparations. It is never the empiric choice.

Choose linezolid when there is VRSA or VRE, or when IV medication for MRSA is not possible. And you need oral MRSA coverage. The other time it is the right answer is on board examinations: when you know you need to use vancomycin, but vancomycin isn't an answer choice AND the indication is NOT a bloodstream infection (must use daptomycin, cannot use linezolid).

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Linezolid's class is "oxazolidinones." You will not hear it pronounced by us. Microsoft Word knows it is a word. We did not.

Tetracyclines

Tetracyclines are the first of three classes of medications that prevent elongation by binding to the A site. Initiation is not interfered with, so the 50s and 30s form, methionine is placed, the tRNA comes into the A site, transfer of amino acid, and translocation happens. BOOM. Drug sees that move and sneaks into the A site before the tRNA. It doesn't matter how long the amino acid chain is, how far along the gene the ribosome is. Translocation reveals the A site, and the drug ninjas the A site. The next amino-acyl-tRNA is prevented from entering the A site. Tetracyclines are the model for this system. Glycylcyclines and the streptogramins are things to know about, but are not used in routine practice. The class of medication is tetracycline. One of the drug's in the class tetracyclines is named tetracycline. All tetracycline antibiotics have the suffix -cycline: doxycycline, tetracycline, and minocycline.

Mechanism. Tetracyclines bind to the **A site** of the **30s ribosome** and prevent the attachment of an incoming aminoacyl-tRNA. This prevents elongation, and is therefore bacteriostatic. There are three tetracyclines that you should know of, though if forced to choose, learn the most about **doxycycline**, as it is the tetracycline most often used. Doxycycline is not hepatically metabolized, but is eliminated in bile, and so is not renally eliminated and so does not require dose adjustment for renal impairment. Doxycycline can achieve therapeutic levels in the CNS and comes in both intravenous and oral form. The other tetracyclines are *minocycline* (gets into CSF, hepatically metabolized, renally cleared) and *tetracycline* (renally cleared, does not achieve CSF concentrations).

Uses. Tetracyclines are used to treat infections with bacteria that hide. They are most commonly used to treat facultative intracellular organisms, and, most importantly, **spirochetes** such as *Borrelia burgdorferi* (Lyme disease) and *Treponema pallidum* (syphilis), and **obligate intracellular organisms** such and *Rickettsia* species (Rocky Mountain spotted fever) and *Chlamydia* species. Doxycycline is also used to treat acne. Another use specifically for doxycycline is the treatment of acute bronchitis in COPD exacerbations, especially when azithromycin (a macrolide) is contraindicated because of prolonged QT.

Side effects. NEVER give tetracyclines to **pregnant women** or **children under 8.** Tetracyclines are **calcium chelators**, which will deposit in calcium-rich areas like bones and teeth. **Deposits in teeth** cause permanent discoloration. **Deposits in bone** impair bone growth. **Eating dairy or calcium** with tetracycline administration prevents its absorption by forming nonabsorbable chelates. This chelation phenomenon also happens with magnesium and iron. So while dairy must absolutely be avoided, it is generally a good idea to counsel the taking of a tetracycline on an empty stomach, with avoidance of milk to be especially emphasized (which someone might drink to swallow the pill).

Resistance. Rather than preventing the entry of the drug into the bacteria, the bacteria develop **efflux pumps** that actively pump the drug out of the cell, preventing its accumulation in the cytosol, preventing the effective dose inside the bacteria.

Glycylcyclines (gly-sill-CYClines)

Tigecycline is the only glycylcycline available. It works just like tetracyclines, except that it can be used to treat **MRSA**, **VRE**, many anaerobic bacteria, and even **ESBL Gram negatives**. However, it does **not treat *Pseudomonas***. You will never choose this drug. It is used almost exclusively as an antibiotic of last resort in intra-abdominal wound infections in postoperative liver transplant patients. It was created to overcome the bacteria that became resistant to tetracyclines via efflux pumps. It worked. Tetracycline-resistant organisms were treated effectively with tigecycline. However, resistance has already been seen against tigecycline. The mechanism? MOAR efflux pumps.

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Streptogramins (Combination of Quinupristin and Dalfopristin)

This is a combination of the drugs quinupristin (30%) and dalfopristin (70%). They are **so toxic** that they are not used unless there is a VRE infection and no other options. Quinupristin prevents elongation similar to macrolides (see below), while dalfopristin disrupts elongation by interfering with the addition of new amino acids to the peptide chain by preventing the incoming amino-acyl-tRNA from interacting with the A site (like tetracyclines). Quinupristin/dalfopristin work from the **50s side.** They are used to treat VRE and VRSA. Streptogramin, quinupristin/dalfopristin, or any permutation resembling them are never the right answer. That is because they cause caustic irritation to veins when delivered through anything not a central line, hepatic catastrophe with cholestatic jaundice, and a mess of CYP450 interactions. Because there are less-toxic options, their use is minimal, and only when there is no other choice.

Chloramphenicol

Chloramphenicol also works by limiting amino acid chain elongation like the drugs listed in the previous sections. However, unlike the previous drugs that block the A site, chloramphenicol binds and **inhibits peptidyl transferase**. It does so while binding to the **50s subunit**. The ribosome will not translocate until peptidyl transferase moves the amino acid chain over and elongation factor gives the thumbs up. If peptidyl transferase is inhibited, and the amino acid chain does move, elongation factor will never give the thumbs up, so all protein synthesis arrests; chloramphenicol is therefore bacteriostatic. This is an antibiotic that is used only with special permission and as a last resort. Knowing it as the only medication that affects peptidyl transferase is one key. The other key is the toxic side effects—grey baby syndrome and dose-dependent bone marrow suppression.

Grey Baby syndrome. Because this antibiotic **mistakes mammalian mitochondrial ribosomes for bacterial ribosomes**, toxic accumulation leads to the failure of human mitochondria to do what they do—make ATP. Chloramphenicol is metabolized (glucuronidated) in the liver and eliminated as a metabolite in the kidneys. Neonates have an incomplete glucuronidation system and incomplete kidneys, both of which lead to **accumulation** of chloramphenicol. When the concentration gets high enough, mitochondria stop making ATP. This presents as poor feeding, then depressed breathing, and resultant cardiovascular collapse results in death. Adults who receive very high doses can exhibit these affects as well, but it is the neonate's impaired hepatic biotransformation and impaired renal elimination that are at greatest risk.

Dose-dependent bone marrow suppression. Because chloramphenicol, at high enough concentrations, can mistake mammalian ribosomes as bacterial, it can cause human cells to stop making protein. Remember, the drug is bacteriostatic, so it will be "translationo-static" in humans. The cells at worst risk are the bone marrow. There is a dose-dependent anemia associated with chloramphenicol. Remove the drug, remove the side effects.

Macrolides

Macrolides are macromolecules—they are enormous. They inhibit protein synthesis, so must be in the cytoplasm. The only way a very large molecule could ever get into the cytoplasm (passing the cell membrane) is to be very **lipophilic**. When identifying macrolides, do NOT look only at the -mycin ending; that is too abundant in multiple drug classes. What you want to look for is the -**thro**- in the middle or the -**thromycin** suffix, as in erythromycin, clarithromycin, and azithromycin. Macrolides bind to the **50s ribosomal subunit** and inhibit **translocation**. They are bacteriostatic.

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DRUG	INDICATIONS	SIDE EFFECTS
Azithromycin	Community-acquired pneumonia with ceftriaxone Acute bronchitis in COPD *Mycobacterium avium-intracellulare* prophylaxis in AIDS CD4 < 50	Prolonged QT, arrythmias Hepatotoxic as eliminated in bile
Clarithromycin	*H. pylori* triple therapy	Renal dose-adjust as eliminated in urine Hepatically metabolized P450 inhibitor
Erythromycin	Not used as an antibiotic, but as a prokinetic agent in delayed gastric emptying (intravenous) If given orally, completely destroyed by stomach acid EXCEPT in newborns with chlamydia eye infxns	Motilin receptor activation, Hepatotoxic as eliminated in bile P450 inhibitor Erythro drops for prophylaxis Erythro systemic for active infection

Table 4.3: Uses of Macrolides
These drugs are effectively entirely different classes. They do not share commonalities other than their mechanism of action—binding to the 50s ribosome and preventing translocation. Being lipophilic, as in, made of fat, it is not surprising that they impact the organ of fatty acid oxidation, fatty acid synthesis, and drug detoxification—the liver. But how they do that, what damage they do, and what they are used to treat vary between them. Learn them as three distinct drugs with a common mechanism of action in regard to the 50s ribosome. Table 4.3 has everything you need. Since they are lipophilic, they can cross the placental barrier and are teratogens.

If nothing else, learn azithro for lungs and causes QT syndrome, clarithro for *H. pylori*, erythro for babies with chlamydia eye infections or gastroparetics in acute crisis.

Clindamycin

Clindamycin works just like macrolides—binding to the **50s ribosomal subunit** and inhibits **translocation**. It even has a name that makes it sound like it is macrolide. It is not a macrolide. Clindamycin comes in intravenous and oral forms, and, unlike the macrolide cousins, is used to **treat MRSA**, **anaerobes,** and strep. We have no good explanation for how that happens, but learn that it can treat community-acquired MRSA (so will be used to treat cellulitis), and is used to treat anaerobic infections outside of the gut. Metronidazole is used to treat anaerobes in the gut and vagina (according to our completed antibiotic ladder in Antibacterials #1: *Introduction to Antibacterials*).

You will choose clindamycin for anaerobic infections such as lung abscesses. You will choose clindamycin in gas gangrene, a soft skin infection caused by anaerobes, because clindamycin stops toxin production. You might choose clindamycin to treat a vancomycin osteomyelitis, because it penetrates the bone well. You might choose clindamycin as a treatment for community-acquired MRSA infections. For the "mights," you should not choose clindamycin.

The only thing you should know about clindamycin other than that it sounds like and works like a macrolide is that it **causes *C. diff* colitis**. It is THE antibiotic with the **highest risk of *C. diff* infection**. If you see clindamycin and diarrhea, the patient has *C. diff*. Purposefully choosing the antibiotic that is known to cause *C. diff* colitis when there is an alternative doesn't sound like a great idea. So, in clinical practice, it is usually NOT the antibiotic of choice.

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